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ON THE INTERCHANGE OF THE LIMBS OF THE CHICK BY TRANSPLANTATION.

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The experiments of Lillie ('04) and of Shorey ('09) have demonstrated that the injury of certain parts, and the removal of organs in the early stages of the embryonic development of the chick are rarely followed by regeneration of the lost part. Lillie found some evidence of the regeneration of the notochord, but a new wing did not develop after removal of the bud. From these experiments he drew the conclusion that the embryo of the chick possesses little more power of regeneration than the adult. Shorey also removed the wing buds of embryos of three to six days' incubation, and although the region healed and development of other parts proceeded normally, the wing buds did not show any sign of regenerative activity.

In the winter of 1908 I undertook a series of experiments, first to find out if the limb buds after removal could be grafted on again, and if so whether a leg bud grafted on the proximal part of the wing would develop into a wing or a leg, and vice versa, if a wing bud when grafted on the proximal part of the leg would develop into a leg or a wing.

Experiments of this kind on the chick are necessarily attended with many difficulties, so that even under the most favorable conditions, the percentage of successful operations is exceedingly small. My results, therefore, are largely negative, but it may be of interest to note what has been accomplished in the hope that more perfect methods will enable some one to obtain more satisfactory results.

Two general methods were followed. In the first series of experiments the eggs were left in the shell. A window was made above the embryo and after the operation it was sealed by the method which I ('98) used in experiments on the primitive streak. This method has since been used by other investigators with success. In the later experiments the eggs were removed from

the shell at the end of the forty-eighth hour of incubation, and during the further development they were kept in porcelain cups in a moist chamber. The latter method was found more practicable, as the first appearance and the subsequent growth of the buds could be observed, and also the changes in the embryo after the operation could be watched from time to time.

The limb buds are not large enough for removal until the beginning of the fourth day, and allowing for the delay in development caused by the operation, at least four additional days of incubation are needed before the wings and legs show marked characteristics. At the end of the fourth day the embryo lies on its left side; for this reason the operations were made on the right side. The buds were removed by means of a curved knife

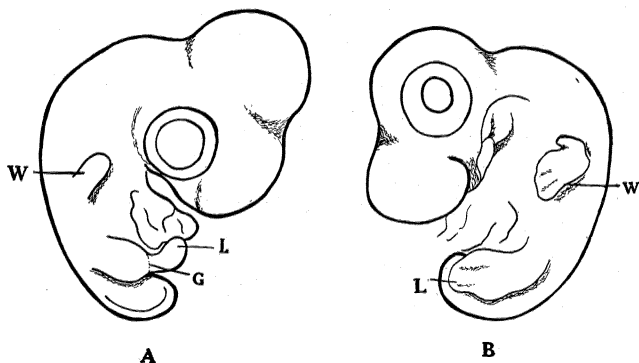


FIG. 1. Two views of a chick on the seventh day. *A*, the right side showing the stump of the wing *W* and the leg *L* with the line of the graft *G*. *B*, the normal side.

made by heating and bending a no. 12 cambric needle into a hook and sharpening it at the curve. The hook was inserted under the bud and drawn up quickly, thus removing the tip with the least possible disturbance to the surrounding parts. The buds were then carried on the needle to the position desired, and held in place for a few minutes until they adhered. The greatest difficulty was met with in the effort to keep the grafts together. Some were fastened by fine glass threads, but this was not found satisfactory as the insertion of the thread tore the tissues. In many of the embryos the grafts came apart, and the buds floated in the albumen or sank in the yolk. Another dif-

difficulty arose through excessive bleeding after the operation. This usually resulted in the death of the embryo.

A brief description of the behavior of one or two of the embryos will suffice to show the general results.

A. The leg and wing buds of an embryo, incubated two days in the shell and then two days in a porcelain cup, were removed. The leg bud was grafted on the proximal part of the wing, and the wing bud on the proximal part of the leg. The embryo was then placed in a moist chamber in the incubator where it was left for three days. On removal it was found that the graft in the wing region had separated, and that the bud on the proximal end of the leg was only partly attached (Fig. 1, *A*). The left side appeared as shown in Fig. 1, *B*. There was no regeneration in the wing region, and no apparent development in the leg region. Sections of this embryo showed the normal limbs to be composed of a homogeneous mass of mesoderm surrounded by

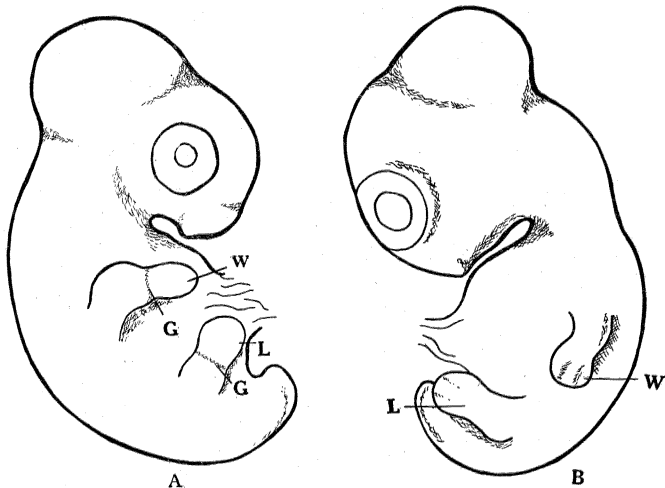


FIG. 2. Two views of a chick on the eighth day. *A*, the right side showing grafts; *B* the normal side.

ectoderm. The limbs on the right side were similar in structure. None of them showed distinguishing characteristics.

B. The same experiment was performed on this embryo as on the one just described. In this case the chick lived four days after the grafts were made. Both unions were complete, as

shown in Fig. 2, *A*, *G*. In Fig. 2, *B*, the left side is indicated. Sections of this chick show concentrated areas where the skeleton is beginning to develop. These areas are indicated in Fig. 3, *A*, *B*, *C*, and *D* by dots. A section of the normal side of the embryo is given in Fig. 3, *D*.

I have not succeeded in keeping any embryos alive beyond the ninth day of incubation, but a normal embryo of this age possesses wings and legs which are readily distinguishable. My

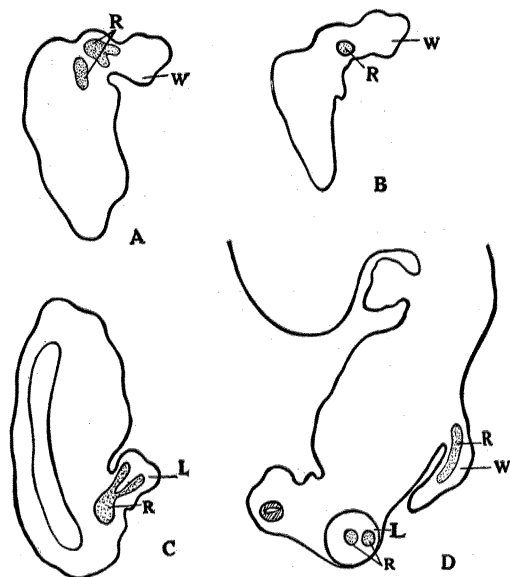


FIG. 3, *A* and *B*. Sections of the grafted wing *W* showing rudiments of the skeleton *R*; *C*, section of the leg *L* with the rudiments of the skeleton *R*; *D*, section of the left side. The leg *L* appears in cross section, the wing *W* in longitudinal section.

results show that the operation greatly retards the development. If the embryos could be kept alive a few days longer it would, no doubt, be possible to determine positively whether or not the grafted tips become a part of the limb to which they are attached irrespective of their former position. The results of my experiments indicate that they do. The failure to keep the embryos alive is probably due to disturbance of the development of the allantois.

In regard to regeneration the results obtained from the removal

of the leg and wing buds fully confirm those of Lillie and Shorey. In no case was the slightest sign of regeneration observed.

SUMMARY.

1. It is possible for chick embryos to develop in porcelain cups in a moist chamber at the proper temperature, up to the ninth day, although the development is delayed.

2. The leg bud when removed may be grafted on the proximal part of the wing and the wing bud may be grafted on the proximal portion of the leg without permanently injuring the embryo.

3. The results indicate that when the tip of a young bud is grafted on the proximal portion of another limb it becomes a part of the appendage to which it is attached instead of retaining the character of the part it is destined to become.

4. No regeneration of the limbs takes place after the removal of the buds.

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